Amendments to the Specification

Please amend the specification as follows:

Please amend paragraph **[0011]** at page 6, as follows:

[0011] The present invention concerns the PIM kinases, (*e.g.*, PIM-1 **(e.g. SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, PIM-2 **(e.g. SEQ ID NO:3 and SEQ ID NO:4)**, and PIM-3 **(e.g. SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:166)**), crystals of the PIM kinases with and without binding compounds, structural information about the PIM kinaes, and the use of the PIM kinases and structural information about the PIM kinases to develop PIM ligands.

Please amend paragraph **[0012]** at page 6, as follows:

[0012] Thus, in a first aspect, the invention provides a method for obtaining improved ligands binding to a PIM kinase (*e.g.*, PIM-1 **(e.g. SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, PIM-2 **(e.g. SEQ ID NO:3 and SEQ ID NO:4)**, PIM-3 **(e.g. SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:166)**), where the method involves determining whether a derivative of a compound that binds to PIM-1 kinase and interacts with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 binds to the PIM kinase with greater affinity or greater specificity or both than the parent binding compound. Binding with greater affinity or greater specificity or both than the parent compound indicates that the derivative is an improved ligand. This process can also be carried out in successive rounds of selection and derivatization and/or with multiple parent compounds to provide a compound or compounds with improved ligand characteristics. Likewise, the derivative compounds can be tested and selected to give high selectivity for the PIM kinase, or to give cross-reactivity to a particular set of targets including

the PIM kinase (*e.g.*, PIM-1), for example, to a plurality of PIM kinases, such as any combination of two or more of PIM-1, PIM-2, and PIM-3.

Please amend paragraph **[0013]** at page 6, as follows:

[0013] The term "PIM kinase" or "PIM family kinase" means a protein kinase with greater than 45% amino acid sequence identity to PIM-1 from the same species, and includes PIM-1 **(e.g. SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, PIM-2 **(e.g. SEQ ID NO:3 and SEQ ID NO:4)**, and PIM-3 **(e.g. SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:166)**. Unless clearly indicated to the contrary, use of the term "PIM kinase" constitutes a reference to any of the group of PIM kinases, specifically including individual reference to each of PIM-1, PIM-2, and PIM-3.

Please amend paragraph **[0022]** at page 8, as follows:

[0022] Reference to particular amino acid residues in PIM-1 polypeptide **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172and SEQ ID NO:173) by** residue number is defined by the numbering provided in Meeker, T. C., Nagarajan, L., ar-Rushdi, A., Rovera, G., Huebner, K., Corce, C. M.; (1987) Characterization of the human PIM-1 gene: a putative proto-oncogene coding for a tissue specific member of the protein kinase family. *Oncogene Res*. 1:87-101, in accordance with the sequence provided in SEQ ID NO: 1. PIM-2 is as described in Baytel et al. (1998) The human Pim-2 proto-oncogene and its testicular expression, *Biochim. Biophys. Acta* 1442,274-285. PIM-3 from rat is described in Feldman, et al. (1998) KID-1, a protein kinase induced by depolarization in brain, *J. Biol. Chem.* 273, 16535-16543; and Kinietzko et al. (1999) Pim kinase expression is induced by LTP stimulation and required for the consolidation of

enduring LTP, *EMBO J.* 18, 3359-3369. (KID-1 is the same as PIM-3.) Human PIM-3 nucleic acid **(SEQ ID NO:165)** and amino acid sequences **(SEQ ID NO:166)** are provided herein.

Please amend paragraph **[0023]** at page 9, as follows:

[0023] In a related aspect, the invention provides a method for developing ligands specific for a PIM kinase, *e.g.*, PIM-1 **(SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, where the method involves determining whether a derivative of a compound that binds to a plurality of kinases has greater specificity for the particular PIM kinase than the parent compound.

Please amend paragraph **[0035]** starting at page 11, as follows:

[0035] Compounds have been identified as PIM-1 inhibitors that had been previously recognized as inhibitors of abl (bcr-abl or c-abl). These compounds include imatinib mesylate (~~Gleevec~~ **GLEEVEC** ™) and related 2-phenylamino pyrimidine compounds, and pyrido-[2,3-d]pyrimidine compounds such as the compound shown in Example 14. Compounds from this group can be used in methods of treating disease associated with PIM-1, e.g., cancers correlated with PIM-1, methods of modulating PIM-1 using these compounds, and methods for developing PIM-1 modulators from derivatives of these compounds, e.g., methods as described herein using crystal structures. Such compounds and methods for preparing them are described in PCT/EP94/03150, WO 95/09847; U.S. Patent 5,543,520; U.S. Patent 5,521,184; U.S. Patent 5,516,775; U.S. Patent 5,733,914; U.S. Patent 5,620,981; U.S. Patent 5,733,913; U.S. Patent 5,945,422; and U.S. Patent 5,945,422. Each of these references is incorporated herein by reference in its entirety.

Please amend paragraph **[0036]** at page 12, as follows:

[0036] Additionally, certain compounds have been identified as molecular scaffolds and binding compounds for PIM-1. Thus, in another aspect, the invention provides a method for identifying a ligand binding to PIM-1 **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, that includes determining whether a derivative compound that includes a core structure selected from the group consisting of Formula I, Formula II, and Formula III as described herein binds to PIM-1 with altered binding affinity or specificity or both as compared to a parent compound.

Please amend paragraph **[0039]** at page 12, as follows:

[0039] In another aspect, structural information about PIM-1 **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)** can also be used to assist in determining a structure for another kinase by creating a homology model from an electronic representation of a PIM-1 structure.

Please amend paragraph **[0040]** starting at page 12, as follows:

[0040] Typically creating such a homology model involves identifying conserved amino acid residues between PIM-1 and the other kinase of interest; transferring the atomic coordinates of a plurality of conserved amino acids in the PIM-1 structure to the corresponding amino acids of the other kinase to provide a rough structure of that kinase; and constructing structures representing the remainder of the other kinase using electronic representations of the structures of the remaining amino acid residues in the other kinase. In particular, coordinates from Table 1 for

conserved residues can be used. Conserved residues in a binding site, *e.g.*, PIM-1 **(SEQ ID NO:1)** residues 49, 52, 65, 67, 121, 128, and 186, can be used.

Please amend paragraph **[0042]** at page 13, as follows:

[0042] The PIM-1 structural information used can be for a variety of different PIM-1 variants, including full-length wild type, naturally-occurring variants (*e.g.*, allelic variants and splice variants), truncated variants of wild type or naturally-occuring variants, and mutants of full-length or truncated wild-type or naturally-occurring variants (that can be mutated at one or more sites). For example, in order to provide a PIM-1 structure closer to a variety of other kinase structures, a mutated PIM-1 **(e.g., mutated SEQ ID NO:1, mutated SEQ ID NO:2, mutated SEQ ID NO:172 or mutated SEQ ID NO:173)** that includes a P123M mutation (proline to mentionine substitution at residue 123) can be used, where the P123M mutation may be the only mutation or there may be a plurality of mutations.

Please amend paragraph **[0043]** at page 13, as follows:

[0043] In another aspect, the invention provides a crystalline form of PIM-1, *e.g.*, having atomic coordinates as described in Table 1 **(SEQ ID NO:172)**. The crystalline form can contain one or more heavy metal atoms, for example, atoms useful for X-ray crystallography. The crystalline form can also include a binding compound in a co-crystal, *e.g.*, a binding compound that interacts with one more ~~more~~ of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)** or any two, any three, any four, any five, any six, or all of those residues, and can, for example, be a compound of Formula I, Formula II, or Formula III. PIM-1 crystals can be in various environments, *e.g.*, in a crystallography plate, mounted for X-ray crystallography, and/or

in an X-ray beam. The PIM-1 may be of various forms, *e.g.*, a wild-type, variant, truncated, and/or mutated form as described herein.

Please amend paragraph **[0045]** at page 14, as follows:

[0045] PIM-1 binding compounds can include compounds that interact with at least one of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)**, or any 2, 3, 4, 5, 6, or 7 of those residues. Exemplary compounds that bind to PIM-1 include compounds of Formula I, Formula II, and Formula III.

Please amend paragraph **[0046]** at page 14, as follows:

[0046] Likewise, in additional aspects, methods for obtaining PIM-1 crystals and co-crystals are provided. In one aspect is provided a method for obtaining a crystal of PIM-1 by subjecting PIM-1 protein at 5-20 mg/ml to crystallization condition substantially equivalent to ~~Hampton Screen~~ **HAMPTON CRYSTAL SCREEN** 1 conditions 2, 7, 14, 17, 23, 25, 29, 36, 44, or 49 for a time sufficient for crystal development. The specified ~~Hampton Screen~~ **HAMPTON CRYSTAL SCREEN** 1 conditions are as follows:

#2 = 0.4 M Potassium Sodium Tartrate tetrahydrate**;**

#7 = 0.1 M Sodium Cacodylate pH 6.5, 1.4 M Sodium Acetate trihydrate**;**

#14 = 0.2 M Calcium Chloride dihydrate, 0.1 M Hepes – Na pH 7.5, 28% v/v Polyethylene glycol 400**;**

#17 = 0.2 M Lithium Sulfate monohydrate, 0.1 M Tris Hydrochloride pH 8.5, 30 % w/v Polyethylene glycol 4000**;**

#23 = 0.2 M Magnesium Chloride hexahydrate, 0.1 M Hepes – Na pH 7.5, 30 % w/v Polyethylene Glycol 400**;**

#25 = 0.1 M Imidazole pH 6.5, 1.0 M Sodium Acetate trihydrate;

#29 = 0.1 M Hepes – Na pH 7.5, 0.8 M Potassium Sodium Tartrate tetrahydrate;

#36 =0.1 M Tris Hydrochloride pH 8.5, 8 % w/v Polyethylene glycol 8000;

#44 = 0.2 M Magnesium Formate**; and**

#49 = 0.2 M Lithium Sulfate monohydrate, 2 % w/v Polyethylene glycol 8000.

Please amend paragraph **[0047]** starting at page 14, as follows:

[0047] Crystallization conditions can be optimized based on demonstrated crystallization conditions. Crystallization conditions for PIM-1 **(e.g., SEQ ID NO:1 and SEQ ID NO:173)** include 0.2 M LiCl, 0.1 M Tris pH 8.5, 5-15% polyethylene glycol 4000; 0.4-0.9 M sodium acetate trihydrate pH 6.5, 0.1 M imidazole; 0.2-0.7 M. sodium potassium tartrate, 00.1 M MES buffer pH 6.5; and 0.25 M magnesium formate. To assist in subsequent crystallography, the PIM-1 can be seleno-methionine labeled. Also, as indicated above, the PIM-1 may be any of various forms, *e.g.*, mutated, such as a P123M mutation **(see SEQ ID NO:1)**.

Please amend paragraph **[0048]** at page 15, as follows:

[0048] A related aspect provides a method for obtaining co-crystals of PIM-1 with a binding compound **(SEQ ID NO:172)**, comprising subjecting PIM-1 protein at 5-20 mg/ml to crystallization conditions substantially equivalent to ~~Hampton Screen~~ **HAMPTON CRYSTAL SCREEN** 1 conditions 2, 7, 14, 17, 23, 25, 29, 36, 44, or 49, as described above in the presence of binding compound for a time sufficient for cystal development. The binding compound may be added at various concentrations depending on the nature of the comound, *e.g.*, final concentration of 0.5 to 1.0 mM. In many cases, the binding compound will be in an organic solvent such as demethyl sulfoxide solution. Some exemplary co-crystallization conditions

include 0.4-0.9 M sodium acetate trihydrate pH 6.5, 0.1 M imidazole; or 0.2-0.7 M. sodium potassium tartrate, 00.1 M MES buffer pH 6.5.

Please amend paragraph **[0049]** at page 15, as follows:

[0049] In another aspect, provision of compounds active on PIM-1 also provides a method for modulating PIM-1 activity by contacting PIM-1 with a compound that binds to PIM-1 and interacts with one more of residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)**, for example a compound of Formula I, Formula II, or Formula III. The compound is preferably provided at a level sufficient to modulate the activity of PIM-1 by at least 10%, more preferably at least 20%, 30%, 40%, or 50%. In many embodiments, the compound will be at a concentration of about 1 μM, 100 μM, or 1 mM, or in a range of 1-100 nM, 100-500 nM, 500-1000 nM, 1-100 μM, 100-500 μM, or 500-1000 μM.

Please amend paragraph **[0053]** at page 16, as follows:

[0053] In a related aspect, the invention provides a method for treating a patient suffering from a disease or condition characterized by abnormal PIM kinase activity, *e.g.*, PIM-1 activity, where the method involves administering to the patient a compound that interacts with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 (**see SEQ ID NO:1;** *e.g.*, a compound of Formula I, Formula II, or Formula III). Similarly, the invention provides a method for treating a patient by administering to the patient a compound that is a 2-phenylaminopyrimidine compound, such as ~~Gleevec~~ **GLEEVEC™ (imatinib mesylate)** or a derivative thereof, or a pyrido-[2,3-d]pyrimidine compound such as the compound shown in Example 14 and derivatives thereof, such as for treating a PIM-1 associated disease such as a PIM-1 associated cancer. Such compounds are described in patents cited above.

Please amend paragraph **[0056]** at page 17, as follows:

[0056] As crystals of PIM-1 have been developed and analyzed, another aspect concerns an electronic representation of PIM-1 **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, for example, an electronic representation containing atomic coordinate representations corresponding to the coordinates listed in Table 1 **(SEQ ID NO:172)**, or a schematic representation such as one showing secondary structure and/or chain folding, and may also show conserved active site residues. The PIM-1 may be wild type, an allelic variant, a mutant form, or a modifed form, *e.g.*, as described herein.

Please amend paragraph **[0057]** at page 17, as follows:

[0057] The electronic representation can also be modified by replacing electronic representations of particular residues with electronic representations of other residues. Thus, for example, an electronic representation containing atomic coordinate representations corresponding to the coordinates listed in Table 1 **(SEQ ID NO:173)** can be modified by the replacement of coordinates for proline at position 123 **(see SEQ ID NO:1)** by coordinates for methionine. Likewise, a PIM-1 representation can be modified by the respective substitutions, insertions, and/or deletions of amino acid residues to provide a representation of a structure for another PIM kinase. Following a modification or modifications, the representation of the overall structure can be adjusted to allow for the known interactions that would be affected by the modification or modifications. In most cases, a modification involving more than one residue will be performed in an iterative manner.

Please amend paragraph **[0059]** starting at page 17, as follows:

[0059] Likewise, in a related aspect, the invention concerns an electronic representation of a portion of a PIM kinase, *e.g.*, PIM-1 **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, e.g., a binding site (which can be an active site), which can include representations of one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)** or residues of the PIM kinase aligning with those PIM-1 residues as shown in the PIM alignment table (Table 2) provided herein. A binding site can be represented in various ways, e.g., as representations of atomic coordinates of residues around the binding site and/or as a binding site surface contour, and can include representations of the binding character of particular residues at the binding site, e.g., conserved residues. As for electronic representations of PIM-1, a binding compound or test compound may be present in the binding site; the binding site may be of a wild type, variant, mutant form, or modified form of PIM-1.

Please amend paragraph **[0062]** starting at page 18, as follows:

[0062] In another aspect, the PIM-1 structural information provides a method for developing useful biological agents based on PIM-1, by analyzing a PIM-1 structure **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)**, to identify at least one sub-structure for forming the biological agent. Such sub-structures can include epitopes for antibody formation, and the method includes developing antibodies against the epitopes, e.g., by injecting an epitope presenting composition in a mammal such as a rabbit, guinea pig, pig, goat, or horse. The sub-structure can also include a mutation site at which mutation is expected to or is known to alter the activity of the PIM-1, and the method includes creating a mutation at that site. Still further, the sub-structure can include an attachment point for attaching a separate moiety, for example, a peptide, a polypeptide, a solid phase material (e.g., beads, gels, chromatographic media, slides, chips, plates, and well surfaces), a linker, and a label (e.g., a direct label such as a

fluorophore or an indirect label, such as biotin or other member of a specific binding pair). The method can include attaching the separate moiety.

Please amend paragraph **[0067]** starting at page 19, as follows:

[0067] Fitting a compound can include determining whether a compound will interact with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)**. Compounds selected for fitting or that are complexed with PIM-1 can, for example, be compounds of Formula I, Formula II, and/or Formula III.

Please amend paragraph **[0068]** at page 20, as follows:

[0068] In another aspect, the invention concerns a method for attaching a kinase binding compound (*e.g.*, a PIM, or PIM-1 binding compound) to an attachment component, as well as a method for indentifying attachment sites on a kinase binding compound. The method involves identifying energetically allowed sites for attachment of an attachment component; and attaching the compound or a derivative thereof to the attachment component at the energetically allowed site. The kinase may be PIM-1 or another kinase, preferably a kinase with at least 25% amino acid sequence identity or 30% sequence similarity to wild type PIM-1 **(SEQ ID NO:1)**, and/or includes conserved residues matching at least one of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1);** i.e., matching any one, any 2, 3, 4, 5, 6, or 7 of those residues).

Please amend paragraph **[0072]** starting at page 20, as follows:

[0072] Another aspect concerns a modified PIM-1 polypeptide **(e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:172 and SEQ ID NO:173)** that includes a P123M modification, and can also include other mutations or other modifications. In various embodiments, the polypeptide includes a full-length PIM-1 polypeptide, includes a modified PIM-1 binding site, includes at least 20, 30, 40, 50, 60, 70, or 80 contiguous amino acid residues derived from PIM-1 including the P123M site, includes any one, any two, or all three of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)**.

Please amend paragraph **[0073]** at page 21, as follows:

[0073] Still another aspect of the invention concerns a method for developing a ligand for a kinase that includes conserved residues matching any one, 2, 3, 4, 5, 6, or 7 of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 **(see SEQ ID NO:1)**, by determining whether a compound of Formula I, Formula II, or Formula III binds to the kinase. The method can also include determining whether the compound modulates the activity of the kinase. In certain embodiments, the kinase has at least 25% sequence identity or at least 30% sequence similarity to PIM-1 **(e.g., SEQ ID NO:1)**.

Please amend paragraph **[0145]** starting at page 33, as follows:

[0145] Table 1 provides atomic coordinates for human PIM-1 **(SEQ ID NO:172)**. In this table and in Table 4, the various columns have the following content, beginning with the left-most column:

ATOM: Refers to the relevant moeity for the table row.

Atom number: Refers to the arbitrary atom number designation within the coordinate table.

Atom Name: Identifier for the atom present at the particular coordinates.

Chain ID: Chain ID refers to one monomer of the protein in the crystal, *e.g.*, chain "A", or to other compound present in the crystal, e.g., HOH for water, and L for a ligand or binding compound. Multiple copies of the protein monomers will have different chain Ids.

Residue Number: The amino acid residue number in the chain.

X, Y, Z: Respectively are the X, Y, and Z coordinate values.

Occupancy: Describes the fraction of time the atom is observed in the crystal. For example, occupancy = 1 means that the atom is present all the time; occupancy = 0.5 indicates that the atom is present in the location 50% of the time.

B-factor: A measure of the thermal motion of the atom.

Element: Identifier for the element.

Please amend paragraph **[0146]** at page 34, as follows:

[0146] Table 2 **<u>(SEQ ID NOS:1-11, respectively, in order of appearance)</u>** provides an alignment of several PIM kinases, including human PIM-1, PIM-2, and PIM-3 as well as PIM kinases from other species. **<u>Sequences from the following species are included in the alignment: Hs, *Homo sapiens*; Mm, *Mus musculus*; Dr, *Danio rerio*; Xl, *Xenopus laevis*; Cc, *Coturnix coturnix*; and Ce, *Caenorhabditis elegans*. Residues with > 90% and > 75% conservations are boxed and boxed with gray background, respectively. Phosphate binding sites are indicated by shaded circles. Residues that are invariably involved in ligand binding are indicated by filled uparrows, whereas residues that can be involved in ligand binding are indicated by open uparrows. The backbone atoms of two residues (indicated by leftarrows) in the hinge region have been shown to make hydrogen bonds to ligands in</u>**

many known kinase/ligand complex structures. Note that PIM family kinases all have Pro as the second residue, resulting in the loss of a hydrogen bond donor.

Please amend paragraph **[0147]** at page 34, as follows:

[0147] Table 3 **(SEQ ID NOS:12-102, 170, 103-116, 171, and 117-164, respectively, in order of appearance)** provides alignments of a large set of kinases, providing identification of residues conserved between various members of the set.

Please amend paragraph **[0148]** at page 34, as follows:

[0148] Table 4 provides atomic coordinates for PIM-1 **(SEQ ID NO:172)** with AMP-PNP in the binding site.

Please amend paragraph **[0149]** at page 34, as follows:

[0149] Table 5 provides the nucleic acid **(SEQ ID NO:165)** and amino acid **(SEQ ID NO:166)** sequences for human PIM-3.

Please amend paragraph **[0152]** at page 35, as follows:

[0152] Specific compounds that are c-abl inhibitors and were discovered to also be inhibitors of PIM-1 include imatinib mesylate (~~Gleevec~~ **GLEEVEC**™) and the compound shown in Example 14. Co-crystal structures **[[f]]** **of** the kinase domain of c-Abl with these two compounds

was described in Nagar et al. (2002) *Cancer Res.* 62:4236-4243. Compounds of these classes, i.e., 2-phenylaminopyrimidine compounds such as ~~Gleevec~~ **GLEEVEC™** or a derivative thereof, of a pyrido-[2,3-d]pyrimidine compound such as the compound shown in Example 14 and derivatives thereof can be used in treating PIM-1 correlated diseases such as PIM-1 correlated cancers, and for developing additional derivative PIM-1 inhibitors. Such compounds are described in the patent publications cited in the Summary herein.

Please amend paragraph **[0170]** at page 40, as follows:

[0170] Exemplary mutations for PIM family kinases include the substitution or of the proline at the site corresponding to residue 123 in human PIM-1 **(SEQ ID NO:1)**. One useful subsitution is a proline to methionine substitution at residue 123 (P123M). Such substitution is useful, for example, to assist in using PIM family kinases to model other kinases that do not have proline at that site. Additional exemplary mutations include substitution or deletion of one or more of PIM-1 residues 124-128 or a residue from another PIM aligning with PIM-1 residues 124-128. For example, a PIM residue aligning with PIM-1 residue 128 **(SEQ ID NO:1)** can be deleted. Mutations at other sites can likewise be carried out, *e.g.*, to make a mutated PIM family kinase more similar to another kinase for structure modeling and/or compound fitting purposes.

Please amend paragraph **[0191]** at page 46, as follows:

[0191] The present invention provides high-resolution three-dimensional structures and atomic structure coordinates of crystalline PIM-1 **(SEQ ID NO:172)** and PIM-1 co-complexed with exemplary binding compounds **(SEQ ID NO:172)** as determined by X-ray crystallography. The specific methods used to obtain the structure coordinates are provided in the examples. The atomic structure coordinates of crystalline PIM-1 are listed in Table 1, and atomic coordinates for

PIM-1 co-crystallized with AMP-PMP are provided in Table 4. Co-crystal coordinates can be used in the same way, *e.g.*, in the various aspects described herein, as coordinates for the protein by itself.

Please amend paragraph **[0242]** at page 60, as follows:

[0242] Binding parameters can be measured using surface plasmon resonance, for example, with a ~~BIAcore~~ **BIOCORE®** chip (Biacore, Japan) coated with immobilized binding components. Surface plasmon resonance is used to characterize the microscopic association and dissociation constants of reaction between an sFv or other ligand directed against target molecules. Such methods are generally described in the following references which are incorporated herein by reference. Vely F. et al., (2000) ~~BIAcore~~ **BIOCORE®** analysis to test phosphopeptide-SH2 domain interactions, *Methods in Molecular Biology*. 121:313-21; Liparoto et al., (1999) Biosensor analysis of the interleukin-2 receptor complex, *Journal of Molecular Recognition*. 12:316-21; Lipschultz et al., (2000) Experimental design for analysis of complex kinetics using surface plasmon resonance, *Methods*. 20(3):310-8; Malmqvist., (1999) BIACORE: an affinity biosensor system for characterization of biomolecular interactions, Biochemical Society Transactions 27:335-40; Alfthan, (1998) Surface plasmon resonance biosensors as a tool in antibody engineering, *Biosensors & Bioelectronics*. 13:653-63; Fivash et al., (1998) BIAcore for macromolecular interaction, Current Opinion in Biotechnology. 9:97-101; Price et al.; (1998) Summary report on the ISOBM TD-4 Workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. *Tumour Biology* 19 Suppl 1:1-20; Malmqvist et al, (1997) Biomolecular interaction analysis: affinity biosensor technologies for functional analysis of proteins, *Current Opinion in Chemical Biology*. 1:378-83; O'Shannessy et al., (1996) Interpretation of deviations from pseudo-first-order kinetic behavior in the characterization of ligand binding by biosensor technology, *Analytical Biochemistry*. 236:275-83; Malmborg et al., (1995) BIAcore as a tool in antibody engineering, *Journal of Immunological Methods*. 183:7-13; Van Regenmortel, (1994)

Use of biosensors to characterize recombinant proteins, *Developments in Biological Standardization*. 83:143-51; and O'Shannessy, (1994) Determination of kinetic rate and equilibrium binding constants for macromolecular interactions: a critique of the surface plasmon resonance literature, *Current Opinions in Biotechnology*. 5:65-71.

Please amend paragraph **[0243]** at page 61, as follows:

[0243] **~~BIAcore~~** **<u>BIOCORE</u>**® uses the optical properties of surface plasmon resonance (SPR) to detect alterations in protein concentration bound to a dextran matrix lying on the surface of a gold/glass sensor chip interface, a dextran biosensor matrix. In brief, proteins are covalently bound to the dextran matrix at a known concentration and a ligand for the protein is injected through the dextran matrix. Near infrared light, directed onto the opposite side of the sensor chip surface is reflected and also induces an evanescent wave in the gold film, which in turn, causes an intensity dip in the reflected light at a particular angle known as the resonance angle. If the refractive index of the sensor chip surface is altered (e.g., by ligand binding to the bound protein) a shift occurs in the resonance angle. This angle shift can be measured and is expressed as resonance units (RUs) such that 1000 RUs is equivalent to a change in surface protein concentration of 1 ng/mm^2. These changes are displayed with respect to time along the y-axis of a sensorgram, which depicts the association and dissociation of any biological reaction.

Please amend paragraph **[0251]** starting at page 62, as follows:

[0251] In spectrofluorometric methods, enzymes are exposed to substrates that change their intrinsic fluorescence when processed by the target enzyme. Typically, the substrate is nonfluorescent and is converted to a fluorophore through one or more reactions. As a non-limiting example, SMase activity can be detected using the **~~Amplex~~** **<u>AMPLEX</u>**® Red reagent

(Molecular Probes, Eugene, OR). In order to measure sphingomyelinase activity using ~~**Amplex**~~ **AMPLEX**® Red, the following reactions occur. First, SMase hydrolyzes sphingomyelin to yield ceramide and phosphorylcholine. Second, alkaline phosphatase hydrolyzes phosphorylcholine to yield choline. Third, choline is oxidized by choline oxidase to betaine. Finally, H_2O_2, in the presence of horseradish peroxidase, reacts with ~~**Amplex**~~ **AMPLEX**® Red to produce the fluorescent product, Resorufin, and the signal therefrom is detected using spectrofluorometry.

Please amend paragraph **[0259]** at page 65, as follows:

[0259] The target molecule can be bound to the scintillator plates by a variety of well known means. Scintillant plates are available that are derivatized to bind to fusion proteins such as GST, His6 **(SEQ ID NO: 167)** or Flag fusion proteins. Where the target molecule is a protein complex or a multimer, one protein or subunit can be attached to the plate first, then the other components of the complex added later under binding conditions, resulting in a bound complex.

Please amend paragraph **[0337]** at page 88, as follows:

[0337] Through the identification of full-length human PIM-3 (hPIM-3**, SEQ ID NO:165 and SEQ ID NO:166**), the invention additionally provides the coding sequence for hPIM-3, thereby allowing cloning, construction of recombinant hPIM-3, production and purification of recombinant hPIM-3 protein, introduction of hPIM-3 into other organisms, and the like.

Please amend paragraph **[0365]** at page 98, as follows:

[0365] The PIM-1 DNA encoding amino acids 1-313 **(SEQ ID NO:1)** and 29- 313 **(SEQ ID NO:173)** were amplified from human brain cDNA (Clonetech) by PCR protocols and cloned into a modified pET 29 vector (Novagen) between NdeI and SalI restriction enzyme sites. The amino acid sequences of the cloned DNA were confirmed by DNA sequencing and the expressed proteins contain a hexa-histidine sequence at the C terminus. The protein was expressed in *E. coli* BL21(DE3)pLysS (Novagen). The bacteria were grown at 22°C in Terrific broth to 1-1.2 OD600 and protein was induced by 1 mM IPTG for 16-18 h. The bacterial pellet was collected by centrifugation and stored at -70°C until used for protein purification. PIM-2 and PIM-3 are cloned similarly.

Please amend paragraph **[0366]** starting at page 98, as follows:

[0366] The bacterial pellet of approximately 250-300g (usually from 16 L) expressing PIM-1 kinase domain (29-313) **(SEQ ID NO:173)** was suspended in 0.6 L of Lysis buffer (0.1 M potassium phosphate buffer, pH 8.0, 10 % glycerol, 1 mM PMSF) and the cells were lysed in a French Pressure cell at 20,000 psi. The cell extract was clarified at 17,000 rpm in a Sorval SA 600 rotor for 1 h. The supernatant was re-centrifuged at 17000 rpm for another extra hour. The clear supernatant was added with imidazole (pH 8.0) to 5 mM and 2 ml of cobalt beads (50% slurry) to each 40 ml cell extract. The beads were mixed at 4°C for 3-4 h on a nutator. The cobalt beads were recovered by centrifugation at 4000 rpm for 5 min. The pelleted beads were washed several times with lysis buffer and the beads were packed on a Biorad disposable column. The bound protein was eluted with 3-4 column volumes of 0.1 M imidazole followed by 0.25 M imidazole prepared in lysis buffer. The eluted protein was analyzed by SDS gel electrophoresis for purity and yield.

Please amend paragraph **[0367]** at page 99, as follows:

[00367] The eluted protein from cobalt beads was concentrated by Centriprep-10 (Amicon) and separated on Pharmacia Superdex 200 column (16/60) in low salt buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM beta mercaptoethanol). The peak fractions containing PIM-1 kinase **(SEQ ID NO:173)** was further purified on a Pharmacia Source Q column (10/10) in 20 mM Tris-HCl pH 7.5 and 14 mM beta mercaptoethanol using a NaCl gradient in an AKTA-FPLC (Pharmacia). The PIM-1 kinase eluted approximately at 0.2 M NaCl gradient. The peak fractions were analyzed by SDS gel electrophoresis and were pooled and concentrated by Centriprep 10. The concentrated PIM-1 protein (usually 50-60 A280/ml) was aliquoted into many tubes (60ul), flash frozen in liquid nitrogen and stored at -70°C until used for crystallization. The frozen PIM-1 kinase still retained kinase activity as concluded from activity assays. PIM-2 and PIM-3 can be purified in the same way with small adjustments to conditions, *e.g.*, elution conditions.

Please amend paragraph **[0369]** at page 100, as follows:

[0369] There are no reports of PIM-1 **(SEQ ID NO:1)** existing in more than one form in human. Analysis of PIM-1 gene sequence reveals that the presence of in-frame stop codons block synthesis of proteins with N terminal extensions. However, the human PIM-2 **(SEQ ID NO:3)** gene contains no in-frame stop codon, based on the reported DNA sequence. Therefore, alternate initiation at an upstream start codon is possible. We have expressed the PIM-2 kinase domain in *E.coli* and purified the protein by the same methods as described for PIM-1 kinase.

Please amend paragraph **[0370]** at page 100, as follows:

[0370] All materials were purchased through Hampton Research, Inc. (Laguna Niguel, CA) unless otherwise noted. PIM-1 protein **(SEQ ID NO:173; see Example 2, paragraphs [0366]-**

[0367]) @ 7 and 14 mg/ml was screened against ~~Hampton Crystal Screen~~ HAMPTON CRYSTAL SCREEN 1 and 2™ kits (HS1 and HS2) and yielded successful crystals growing in at least 10 conditions from HS1 alone. Crystals were grown initially using sitting drops against the Hampton screening conditions set in Greiner 96 well CrystalQuick crystallization plates with 100 ul reservoir and 1 ul protein + 1 ul reservoir added per platform (1 of 3 available). Conditions from ~~Hampton Screen~~ HAMPTON CRYSTAL SCREEN 1 yielded obvious protein crystals in conditions: #2, 7, 14, 17, 23, 25, 29, 36, 44, and 49. These crystals were grown at 4°C, and grew in size to varying dimensions, all hexagonal rod shaped and hardy.

Please amend paragraph **[0371]** at page 100, as follows:

[0371] Crystals of larger dimensions, 100 **[[uM]]** **µM** wide x 400 **[[uM]]** **µM** long, were then grown in larger drop volumes and in larger dimension plates. Refined grids were performed with both hanging and sitting drop methods in ~~VDX plates~~ **VDX PLATEs™** (cat. # HR3- 140) or CrysChem™ plates (cat. # HR3-160). There appeared to be no obvious difference of crystal size or quality between the two methods, but there was a preference to use hanging drops to facilitate mounting procedures.

Please amend paragraph **[0391]** starting at page 104, as follows:

[0391] Such binding assays can be performed in a variety of ways, including a variety of ways known in the art. For example, competitive binding to PIM-1 can be measured on Nickel-~~FlashPlates~~ **FLASHPLATEs®**, using His-tagged PIM-1 (~ 100 ng) and ATPγ[^{35}S] (~ 10 nCi). As compound is added, the signal decreases, since less ATPγ[^{35}S] is bound to PIM1 which is proximal to the scintillant in the ~~FlashPlate~~ **FLASHPLATE®**. The binding assay can be performed by the addition of compound (10 µl; 20 mM) to PIM-1 protein (90 10 µl) followed by

the addition of ATPγ[^{35}S] and incubating for 1 hr at 37°C. The radioactivity is measured through scintillation counting in Trilus (Perkin-Elmer).

Please amend paragraph **[0395]** at page 105, as follows:

[0395] Inhibitory or exhitory activity of compounds binding to PIM-1 **(SEQ ID NO:1)** was determined using the kinase activity assay described in the detailed description.

Please amend paragraph **[0396]** at page 105, as follows:

[0396] Exemplary compounds within Formula I, Formula II, and Formula III were assayed for inhibitory activity with PIM-1 **(SEQ ID NO:1)**. The ability to develop ligands is illustrated by 2 compounds from the quinolinone molecular scaffold group (Formula III). A compound with R1, R2, R3, R4, R5, and R6 = H, had 100% inhibition of PIM-1 at 200 μM concentration, while a compound with R1 = phenyl group, R2, R3, R5, and R7 = H, and R4 = OCF_3, had only 3% inhibition of PIM-1 at 200 μM.

Please amend paragraph **[0412]** at page 111, as follows:

[0412] The Rat PIM-3 sequence (AF086624) was used to query the public human EST database. Two human EST clones were found with high homology to the rat sequence. EST # AL530963 from brain-derived neuroblastoma cells encodes the N-terminal portion, and EST # BG681342 from skin-derived squamous cell carcinoma cells encodes the C-terminal portion. On the basis of these EST sequence, two oligonucleotides PIM-3S (5'-GCAGCCACATATGGCGGACAAGGAGAGCTTCGAG-3') **(SEQ ID NO: 168)** and PIM-3A

(5'-TGCAGCGTCGACCAAGCTCTCGCTGCTGGACGTG-3') **(SEQ ID NO: 169)** were designed and amplify the kinase domain by PCR reaction from human EST clone # BF204865, which seemed to encode the full length human PIM-3 protein. The PCR products were subcloned into modified pET29a vector, in frame with a carboxy-terminal His tag for bacterial expression. His6-tagged PIM-3 proteins **(SEQ ID NO: 167)** were expressed and purified as described [[in]] **for** PIM-1. The nucleotide sequence encoding human full length PIM-3 protein is attached as well as the amino acid sequence as Table 5.

Please amend the heading to Example 14 after paragraph **[0425]** at page 113, as follows:

Example 14: Inhibition of PIM-1 by ~~Gleevec~~ GLEEVEC™ (imatinib mesylate) and other brc-abl inhibitors

Please amend paragraph **[0426]** at page 113, as follows:

[0426] Consistent with the identification of PIM-1 as a dual activity protein kinase, it was discovered that imatinib mesylate (~~Gleevec~~ GLEEVEC™) and other inhibitors of brc-abl are also inhibitors of PIM-1. Therefore, activity of ~~Gleevec~~ GLEEVEC™ and the following compound was determined.

Please amend paragraph **[0427]** at page 114, as follows:

[0427] Using the PY20 ~~AlphaScreen~~ ALPHASCREEN® kit (Packard BioScience) in accordance with manufacturer**'s** instructions, it was found that ~~Gleevec~~ GLEEVEC™ had an IC_{50} of 80 nM for PIM-1, and the above compound had an IC_{50} of 10 nM; both approximately the

same as for abl. These tests demonstrate that these compounds are potent inhibitors of PIM-1, and can be used for treatment of PIM-1 associated diseases, such as PIM-1 associated cancers.